

A NEW SPECIES OF *DISTICHLIS* (POACEAE, CHLORIDOIDEAE)
FROM BAJA CALIFORNIA, MEXICO

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ABSTRACT

Based upon a specimen first collected by S. N. Stephenson, a new grass species, ***Distichlis bajaensis*** H. L. Bell, is described. Stephenson hypothesized that this specimen was a hybrid between *D. littoralis* and *D. spicata*. Analyses of sequences of nuclear internal transcribed spacer (ITS) and chloroplast *ndhF* and *trnL-trnF* and an examination of gross morphology, blade and lemma micromorphology, and blade transectional anatomy demonstrate that this grass is a new species that may be sister to the remaining *Distichlis*. The blades of ***D. bajaensis*** are yellow-green; those of *D. littoralis* and *D. spicata* are blue-green. ***Distichlis bajaensis*** can be distinguished from *D. littoralis* by its exserted inflorescences with glumes present and from *D. spicata* by its short (0.8–1.5 cm) blades with a bend toward the adaxial side. At and distal to the bend, there are antrorse hairs along the medial vascular bundle. ***Distichlis bajaensis*** is known from a single large population growing along alkaline seeps in Arroyo Rosarito in Baja California, Mexico.

RESUMEN

Se describe como especie nueva de las gramíneas a ***Distichlis bajaensis*** H.L. Bell, basada en un espécimen colectado por la primera vez por S. N. Stephenson. Stephenson postuló que este espécimen era un híbrido de *D. littoralis* y *D. spicata*. Los análisis de secuencias de ADN nuclear (ITS) y del cloroplasto (*ndhF* y *trnL-trnF*), así como los estudios de morfología general, micromorfología (lema y lámina) y anatomía (hoja), demuestran que esta gramínea es una especie nueva y que puede ser hermana a las especies restantes de *Distichlis*. Las hojas de ***D. bajaensis*** son amarillentos verdes pero estas de *D. littoralis* and *D. spicata* son azulinos verdes. Es posible diferenciar ***D. bajaensis*** de *D. littoralis* por las inflorescencias exsertas con glumas y de *D. spicata* por las hojas cortas (0.8–1.5 cm) con una curva hacia la cara abaxial. Hay pelos antrorsos a lo largo del nervio central antes del medio. ***Distichlis bajaensis*** se conoce de una sola población grande que crece a lo largo de filtrars alcalinas en la localidad de Arroyo Rosarito, Baja California, México.

Key Words: Baja California, Chloridoideae, *Distichlis*, halophytic grass.

A putative hybrid between *Monanthochloë littoralis* Engelm. and *Distichlis spicata* (L.) Greene from Baja California, Mexico was reported by Stephenson (1971). The putative hybrid resembled *M. littoralis* in vegetative morphology and *D. spicata* in inflorescence structure. *Monanthochloë littoralis* is distributed in coastal regions of subtropical Mexico and USA with one inland population known from Coahuila, Mexico. *Distichlis spicata* has a much broader distribution in coastal and inland North and South America.

Recent work has placed *Monanthochloë* into synonymy with *Distichlis* based upon anatomical, morphological, and molecular evidence (Bell and Columbus 2008). Thus, *M. littoralis* is hereafter referred to as *D. littoralis* and the putative hybrid is considered interspecific. The present study was undertaken to determine if the population from Stephenson (1971) was still extant and to test whether the plants belonging to this population are hybrids, as Stephenson hypothesized.

A hypothesis of hybrid origin predicts that incongruence may be observed between phylog-

enies derived from nuclear (biparentally inherited) and chloroplast (uniparentally inherited) DNA sequences (McDade 1992; Rieseberg et al. 1996 and refs. therein; Blattner 2004; Jakob and Blattner 2006). To test this hypothesis, I present new sequence data derived from both nuclear and chloroplast genomes. These data are added to existing matrices from Bell (2007) and Bell and Columbus (2008). In addition, whole plant morphology, micromorphology of the abaxial surfaces of blades and lemmas, and blade transectional anatomy of the putative hybrid plants were studied and compared to other species of *Distichlis*.

METHODS

During the springs of 2008 and 2009, extensive searches for historical populations of the putative hybrid were conducted near El Nuevo Rosarito in Baja California, Mexico. Observations were made of the growth habit and site conditions. Leaf material was dried in silica gel for DNA analysis, blades, culms, and spikelets were

preserved in FPA (formalin:propionic acid:ethanol, 1:1:18) for anatomical investigations, and pressed, dried herbarium specimens were prepared for morphological studies. For the remainder of this report, the putative hybrid will be referred to as Baja grass.

Genomic DNA was extracted from leaf tissue from *Stephenson 68-304a* (MSC 216526) and freshly collected material (*Bell 458*, RSA 754084) using DNeasy Plant Mini Kits (Qiagen, Valencia, CA). Sequences of the nuclear ribosomal internal transcribed spacer (ITS) as well as chloroplast *ndhF* and *trnL-trnF* were amplified using primers and protocols described in Bell and Columbus (2008). In order to detect possible allelic variation in ITS, the amplification product was cloned using a TOPO TA kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Ten colonies per sample were screened. Cycle sequencing was conducted at Rancho Santa Ana Botanic Garden on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA) following the protocols of Bell and Columbus (2008).

Sequences were assembled, edited, and incorporated into existing alignments from Bell (2007) and Bell and Columbus (2008). The same out-group taxa used in Bell and Columbus (2008) (*Allolepis texana* (Vasey) Soderstr. & H. F. Decker; *Bouteloua dactyloides* (Nutt.) Columbus; *Eragrostis obtusiflora* (E. Fourn.) Scribn.; *Jouvea pilosa* (J. Presl) Scribn.) were employed in this study. Maximum parsimony (MP) and Bayesian inference (BI) analyses were conducted on the ITS and combined chloroplast (*ndhF* + *trnL-trnF*) data sets using PAUP* (Swofford 2002) and MrBayes vers. 3.0b4 (Huelsenbeck and Ronquist 2001) using the search parameters outlined in Bell and Columbus (2008). In addition, ITS 1 and ITS 2 were analyzed separately (Yokota et al. 1989; Liu and Schardl 1994; Mai and Coleman 1997). Branch support was assessed via posterior probabilities (PP), parsimony bootstrap (BS), and Bremer Support Values (BSV) (Bremer 1988) following Bell and Columbus (2008). Sequences generated during this study were submitted to GenBank and accession numbers are given in Appendix 1.

Abaxial surfaces of leaf blades and lemmas were observed following the methods of Bell and Columbus (2008). Transectional anatomy of leaf blades was examined following Columbus (1999). Descriptive terminology follows Ellis (1976) for anatomy and Ellis (1979) for morphology.

RESULTS

Collection Site

As described by Stephenson (1971), I found Baja grass growing along alkaline seeps in

Arroyo Rosarito adjacent to Mexico Hwy 1, southwest of El Nuevo Rosarito, approximately 100 km north of the border with Baja California Sur. Coordinates of the collection site are 28°43'36"N 114°43'17"W.

Baja grass was one of the dominant species at the site and one of the few grasses present although some *D. spicata* was noted also. No *D. littoralis* was observed. Stephenson found fragments of both male and female plants at the heavily grazed site in 1968; only male plants were located during my extensive searches. Burros and cattle were observed in the area, but the population was not heavily grazed during the time of my collections in 2008 and 2009. Morphological features of the earlier collection (*Stephenson 68-304a*, MSC 216526), e.g., exerted inflorescences, spikelets with glumes, suggested affinities to *D. spicata*. However, in the field, its growth habit resembles that of *D. littoralis*; thus, it is clear why Stephenson would have considered *D. littoralis* to be a possible parent. Like *D. littoralis*, Baja grass possesses stolons and frequently grows up through (as on a trellis) adjacent plants such as species of *Juncus* and *Lycium*. The leaves of both *D. littoralis* and *D. spicata* are usually dark blue-green; those of Baja grass are yellowish-green.

DNA Sequence Analysis

Nine of ten cloned ITS sequences from Baja grass (*Stephenson 68-304a*) were identical; the tenth sequence differed by a single base pair. Sequences from recently collected material (*Bell 458*) of ITS (to the group of nine) and *ndhF* were identical to those generated from *Stephenson 68-304a*; only sequences from *Stephenson 68-304a* (including *trnL-trnF*) were used in the analyses. Descriptive statistics for the MP analyses are given in Table 1. Both specimens of Baja grass (*Stephenson 68-304a* and *Bell 458*) showed a single unique indel, a three base pair repeat in ITS.

The trees with the highest log-likelihood value are shown in Figure 1 (ITS) and Figure 2 (combined chloroplast). In the ITS analyses, Baja grass is supported as sister to all other *Distichlis* (BS = 91%, PP = 1.00, BSV = 4). In the combined chloroplast tree, Baja grass is retrieved in a polytomy with all other *Distichlis* species with good support for the clade (BS = 99%, PP = 1.00, BSV = 7). When ITS 1 and 2 were analyzed separately, the topological position of Baja grass changes (data not shown). With ITS 1 (in the MP strict consensus tree), Baja grass resolves as sister to *D. laxiflora* and *D. scoparia*; with ITS 2, it resolves as sister to the *D. spicata* clade. However, neither of these positions was supported. In addition, when an ITS sequence from the Baja grass was aligned and analyzed with a dataset

TABLE 1. DESCRIPTIVE STATISTICS FOR MAXIMUM PARSIMONY ANALYSES. MP = maximum parsimony, PIC = parsimony informative characters, CI = consistency index, RI = retention index.

Region	Aligned length	% missing data	# of MP trees	MP tree length	PIC	CI	RI
ITS	641	0	26	500	148	0.76	0.82
<i>ndhF</i> + <i>trnL-trnF</i>	2111 + 1040	0.1	209	275	84	0.83	0.86

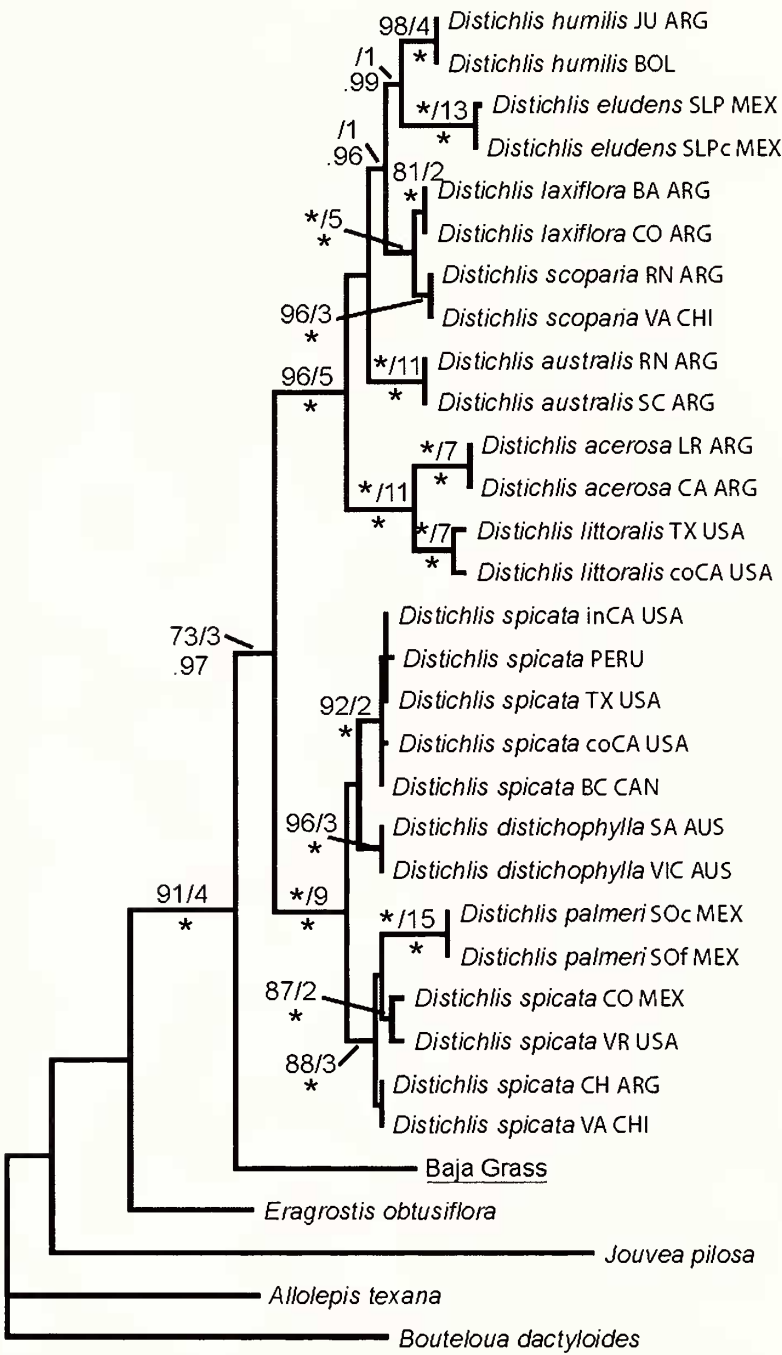


FIG. 1. Tree with the highest log-likelihood score from Bayesian analysis of ITS. Bootstrap values followed by Bremer support are given above the branches, and below are posterior probabilities. An asterisk indicates 100% bootstrap or 1.00 posterior probability. Branches marked with an arrow collapse in the strict consensus from parsimony analysis. Geographical abbreviations are as follows: ARG = Argentina (BA = Buenos Aires, CA = Catamarca, CH = Chubut, CO = Córdoba, JU = Jujuy, LR = La Rioja, RN = Rio Negro, SC = Santa Cruz); AUS = Australia (SA = South Australia, VI = Victoria); BOL = Bolivia; BC CAN = British Columbia, Canada; CHI = Chile (AN = Antofagasta, VA = Valparaiso); MEX = Mexico (CO = Coahuila, SLP = San Luis Potosí, SO = Sonora); USA (CA = California, TX = Texas, VR = Virginia).

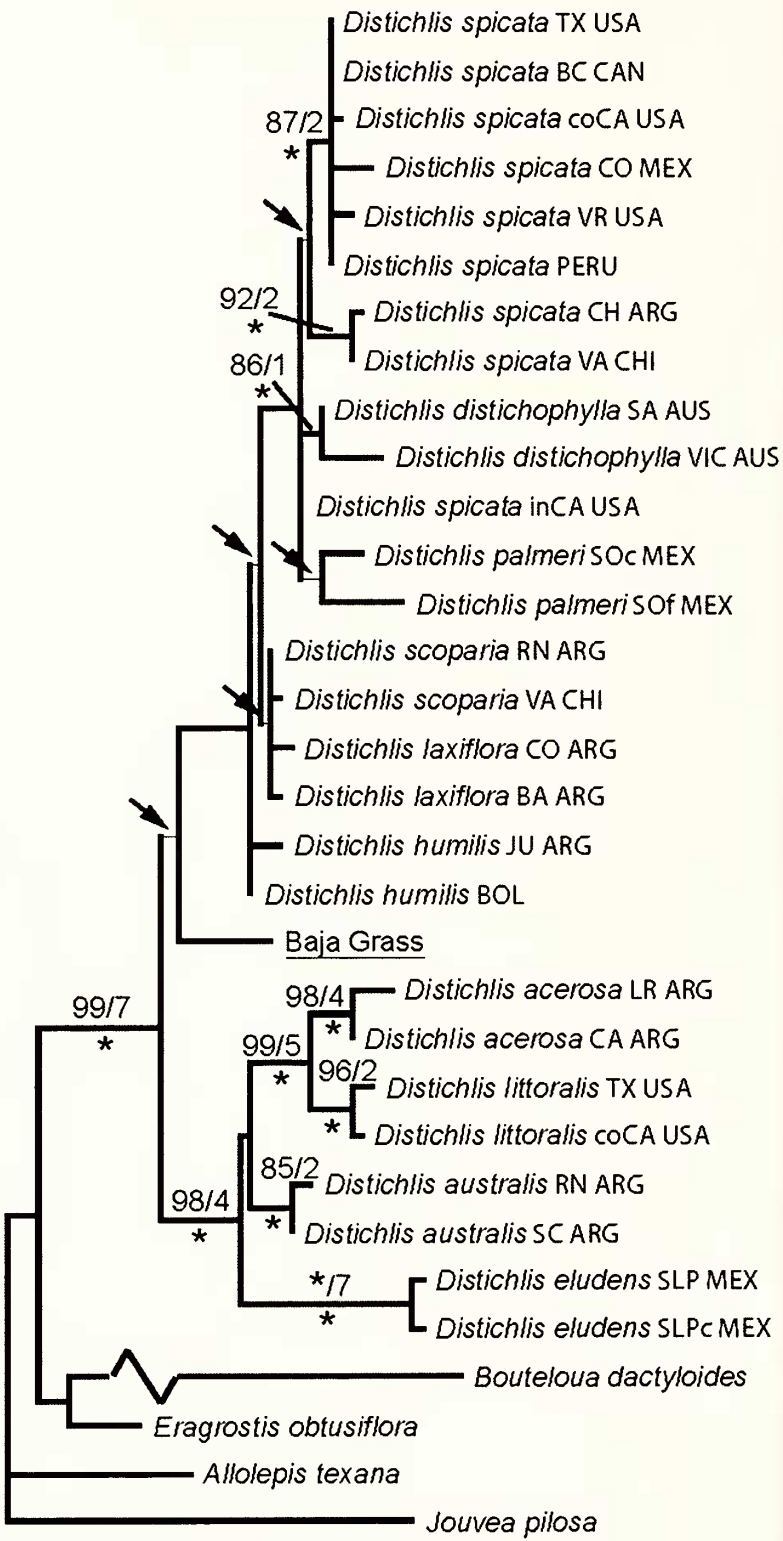


FIG. 2. Tree with the highest log-likelihood score from Bayesian analysis of combined chloroplast data set (*ndhF* + *trnL-trnF*). Bootstrap values followed by Bremer support are given above the branches, and below are posterior probabilities. An asterisk indicates 100% bootstrap or 1.00 posterior probability. Branches marked with an arrow collapse in the strict consensus from parsimony analysis. Geographical abbreviations are the same as in Fig. 1.

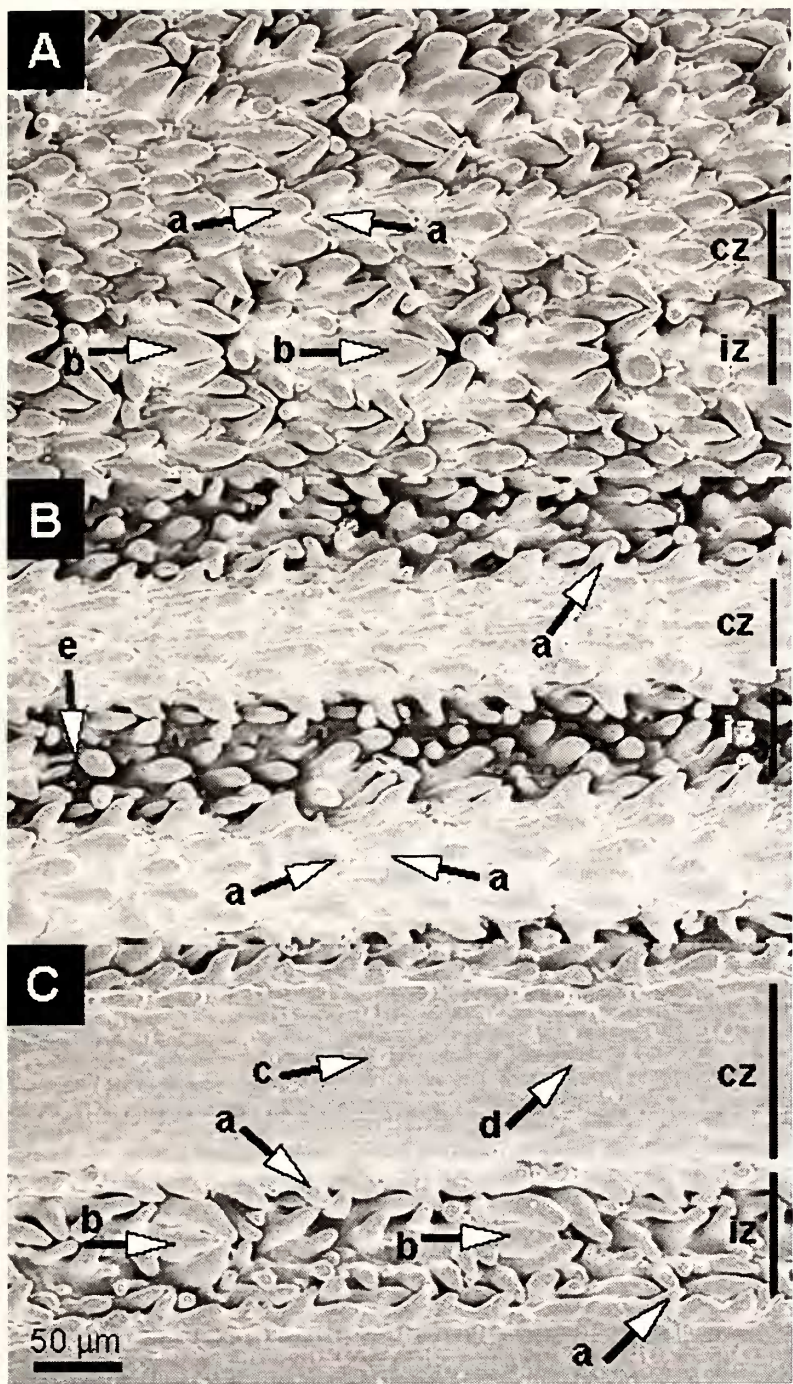


FIG. 3. Comparisons of abaxial blade surfaces; A. Baja grass (Bell 458), B. *Distichlis spicata* (Bell 231), C. *Distichlis littoralis* (Bell 260). a = papilla, b = clustered papillae, c = short cell, d = long cell, e = microhair, cz = costal zone, iz = intercostal zone. Scale bar applies to A, B, and C.

derived from 84 chloridoid genera (Bell 2007), it resolved as sister to *Distichlis* (data not shown).

Micromorphology

Abaxial surfaces of leaf blades of Baja grass are highly papillate making it difficult to observe features such as long and short cells, microhairs and stomates (Fig. 3A). In the costal zones of blades of Baja grass and *D. spicata*, there are regular pairs of large and small papillae (Fig. 3A, B). In intercostal zones of Baja grass and *D. littoralis*, papillae form complexes associated with microhairs (Fig. 3A, C). In Baja grass and species of *Distichlis*, stomates occur in two files along each edge of the intercostal zone; stomates are frequently obscured by complexes of papillae making them difficult to observe from a surface

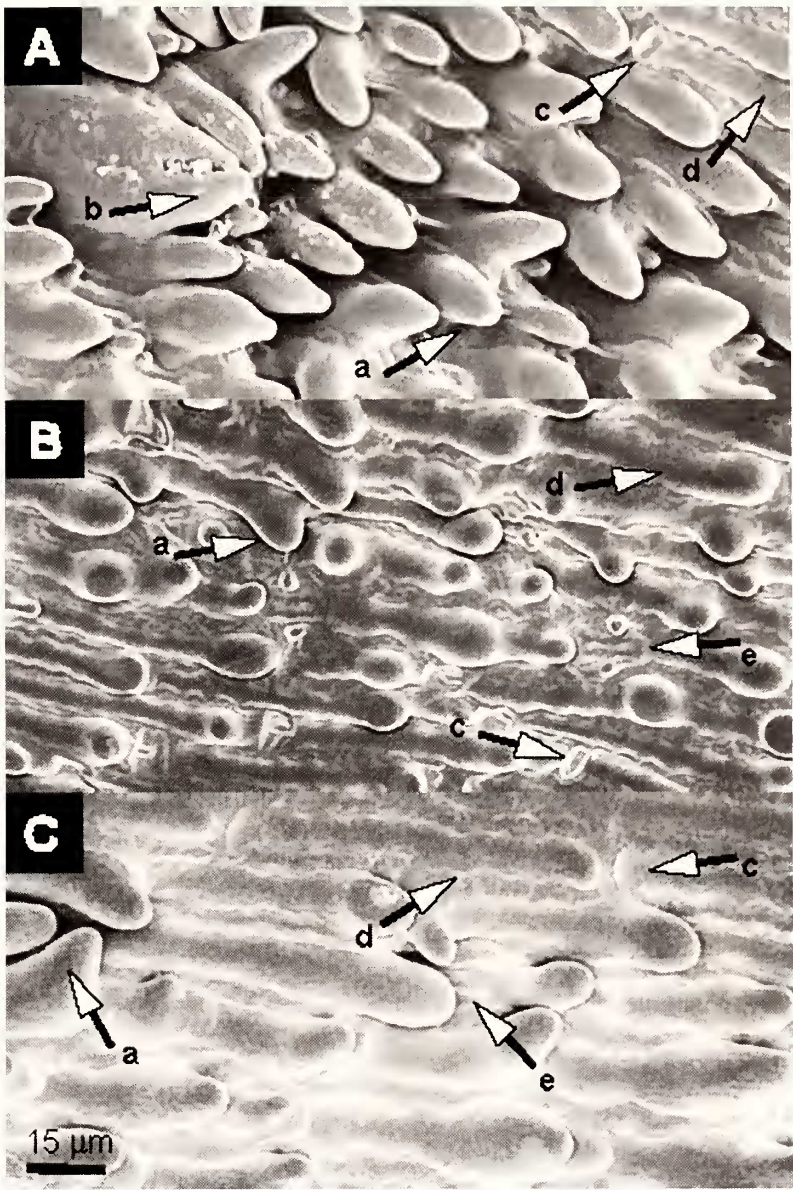


FIG. 4. Comparisons of abaxial surfaces of lemmas; A. Baja grass (Bell 458), B. *Distichlis spicata* (Bell 277), C. *Distichlis littoralis* (Bell 260). a = papilla, b = clustered papillae, c = short cell, d = long cell, e = stomate. Scale bar applies to A, B, and C.

view. Stomates of Baja grass and *Distichlis* have dome shaped subsidiary cells.

Abaxial surfaces of lemmas of Baja grass have many papillae that obscure features such as microhairs and stomates (Fig. 4). There are many complexes of papillae similar to those found on species of *Distichlis*. Microhairs and stomates appeared to be more sparse on lemmas of Baja grass than in *Distichlis* but they may be hidden by papillae.

Anatomy

Blade transectional anatomy of Baja grass is similar to that of *Distichlis* species (Fig. 5). The outline of the blade transection is broadly U-shaped. There are adaxial furrows between all vascular bundles to a depth of about half of the blade thickness. Furrows are absent or shallow on the abaxial side. Blades possessed about 14 total vascular bundles, three of which were 1st order. Examination of species of *Distichlis* found from 18–24 (7–9 1st order) vascular bundles in blades of *D. spicata* and 9 (3 1st order) in *D.*

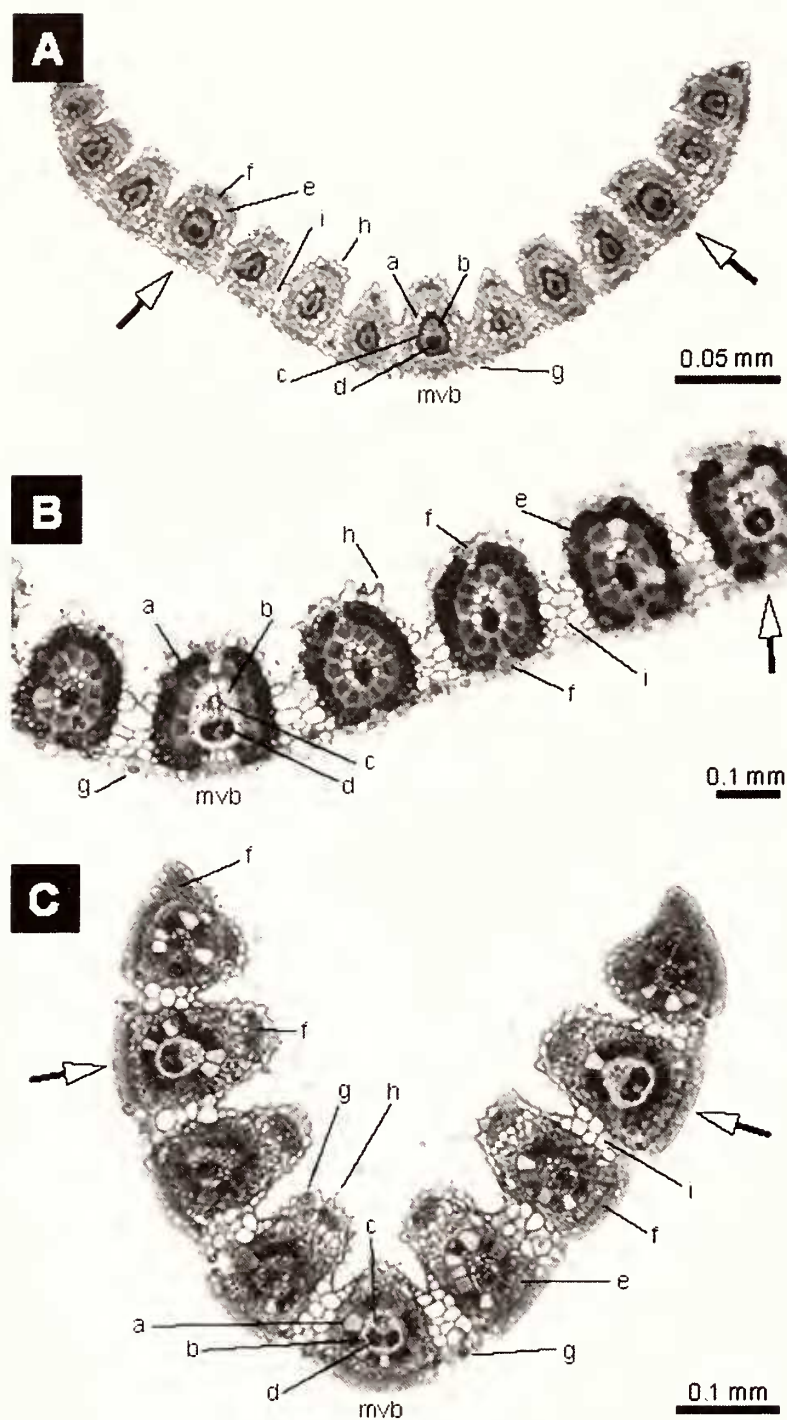


FIG. 5. Comparison of blade anatomy; A. Baja grass (Bell 458), B. *Distichlis spicata* (Bell 375), C. *Distichlis littoralis* (Bell 260). a = outer bundle sheath, b = inner bundle sheath, c = xylem, d = phloem, e = mesophyll, f = sclerenchyma, g = microhair, h = papillae, i = colorless cells.

littoralis (Bell and Columbus 2008). Second order vascular bundles form a regular arrangement between the 1st order bundles; a single 3rd order bundle is found at each margin. Sheath cells in all vascular bundles are elliptical in shape. The outline of 3rd order bundles is round and that of 1st and 2nd order bundles are elliptical. First order vascular bundles have a continuous double sheath that is not interrupted and lacks extensions. Phloem is directly adjacent to the inner sheath, and metaxylem is narrow. Walls of the inner sheath are thickened. Chloroplasts are centripetally arranged in the outer sheath cells. Very narrow strands of sclerenchyma are found on both adaxial and abaxial sides of most vascular bundles, and a small sclerenchyma cap occurs at the margins. Mesophyll forms a single layer of radially arranged cells. Colorless cells form uni- to multiseriate columns between all vascular bundles. Bulliform cells are associated with colorless cells at the base of furrows. Other epidermal cells are small and have numerous papillae on both surfaces. First order vascular bundles of Baja grass show Kranz anatomy of the type that predicts NAD-ME C_4 photosynthesis (Prendergast and Hattersley 1987).

Bicellular microhairs of Baja grass are dumb-bell or flask shaped, with a portion of the basal cell sunken below the epidermis into mesophyll or colorless cells (Fig. 6).

DISCUSSION

Analyses of molecular data do not support the hypothesis that Baja grass is a hybrid between *D. littoralis* and *D. spicata* (McDade 1992; Rieseberg et al. 1996). In both ITS and combined chloroplast (*ndhF* + *trnL-trnF*) analyses, Baja grass does not group with any other species but is supported as sister to or a member of *Distichlis* (Figs. 1 and 2). However, three South American endemics, *D. humilis*, *D. laxiflora*, and *D. scoparia*, are resolved

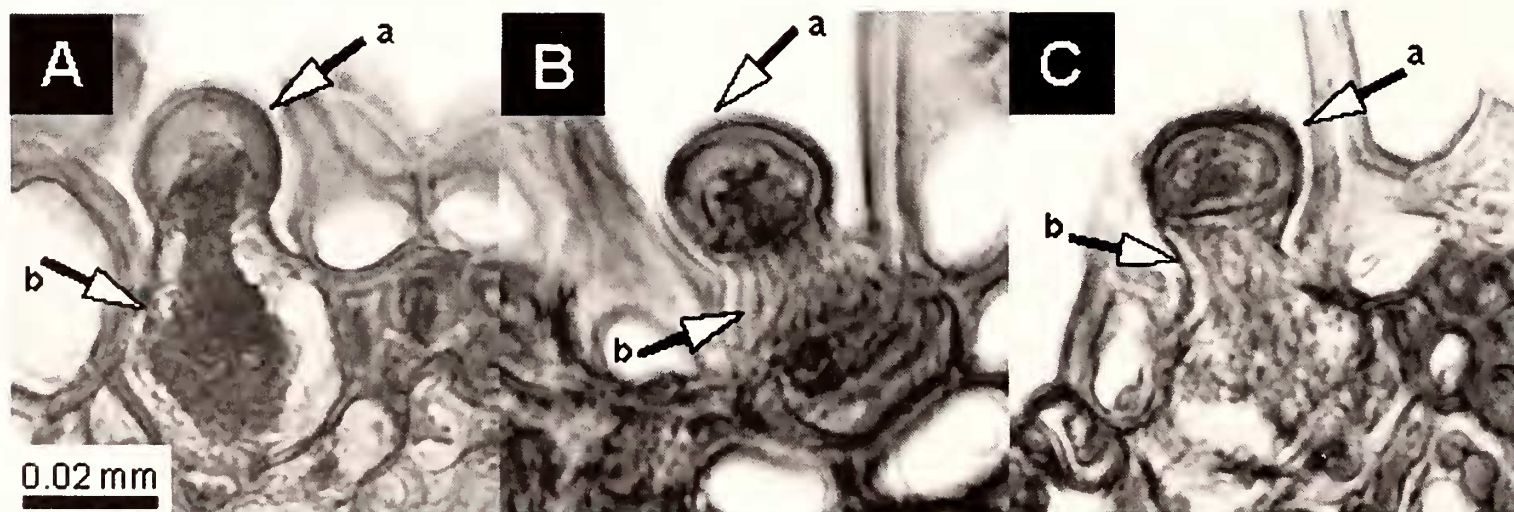


FIG. 6. Bicellular microhairs, A. Baja grass (Bell 458), B. *Distichlis spicata* (Bell 231), C. *Distichlis littoralis* (Bell 260). a = distal cell, b = basal cell. Scale bar applies to A, B, and C.

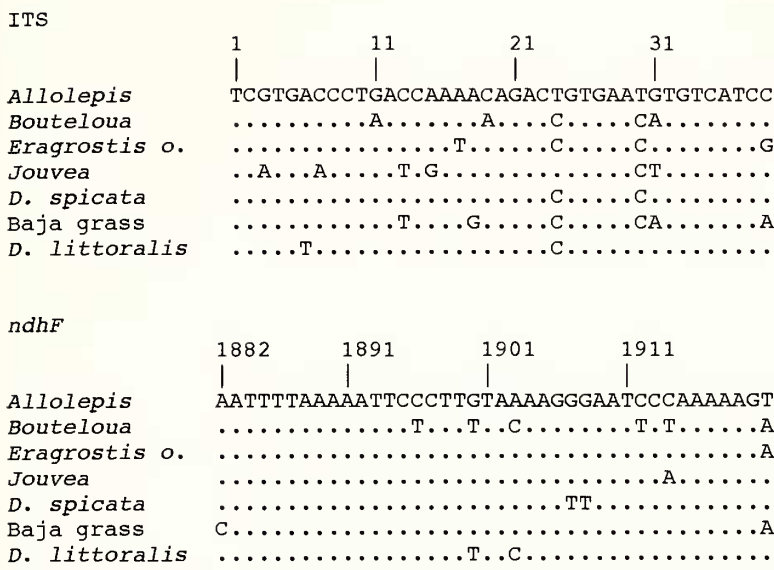


FIG. 7. Patterns of variation in two sections from this study's molecular data sets. Top, the beginning of nuclear ITS; bottom, a relatively variable region of *ndhF*. The first four taxa are the outgroup for this study; they are followed by *D. spicata* (Bell 231), Baja grass (Stephenson 68-304a), and *D. littoralis* (Bell 260).

in conflicting positions by nuclear and chloroplast markers demonstrating that there is adequate signal in these datasets to detect potential reticulation.

A visual comparison of sequence segments from ITS and *ndhF* (Fig. 7) does not reveal the additive pattern that would be predicted if Baja grass were a hybrid between *D. littoralis* and *D. spicata*. If Baja grass were a relatively recent hybrid I would expect to see polymorphisms that were compatible with derivation from *D. littoralis* or *D. spicata*. If the hybridization event occurred in the distant past so that homogenization of ITS alleles had taken place (as is indicated by the finding of nine identical clones), then I would expect that sequences of Baja grass would resemble one or the other of the putative parents. If Baja grass were a hybrid, I would expect that chloroplast sequences would be the same or highly similar to one of the putative parents. As seen in Fig. 7, these are not the patterns that are observed. Variation in sequences from Baja grass does not suggest derivation from either *D. littoralis* or *D. spicata*.

Baja grass has the same blade organization as species of *Distichlis* (Fig. 4). Blades are U-shaped, with vascular bundles separated by furrows and columns of colorless cells. There are few 1st order vascular bundles with narrow xylem elements. There is some variation in the amount of sclerenchyma but its distribution is similar. Dumb bell or flask shaped microhairs are found in Baja grass and all species of *Distichlis* as well as *Eragrostis obtusiflora* and a few other more distantly related halophytic chloridoids. There is evidence that these microhairs are the site of salt secretion in halophytic chloridoids (Oross and Thomson 1982; Amarasinghe and Watson 1988; Warren and Brockelman 1989;

Ramadan 2001; Bell and O'Leary 2003). Salt crystals have been observed on the surface of Baja grass blades.

Habitat preferences and anatomical and morphological similarities of the Baja grass to other *Distichlis* species support its inclusion as a new species within the genus. All *Distichlis* species occur in saline or alkaline habitats, are dioecious, have multi-nerved lemmas, Kranz anatomy that predicts NAD-ME type C₄ photosynthesis, numerous papillae on blade surfaces, bulbous bicellular microhairs, columns of colorless cells between vascular bundles, narrow metaxylem elements in 1st order vascular bundles, and relatively few 1st order vascular bundles per blade. Based upon these shared characters and the molecular evidence, this grass is described as a new species of *Distichlis*.

TAXONOMY

Distichlis bajaensis H. L. Bell, sp. nov. (Fig. 8). — Type: MEXICO, Baja California, Municipio de Ensenada, salt marsh in arroyo 1 km SW of Rosarito, area dominated by juncus and salt grasses, heavily grazed by burros and goats, October 1968, *Stephenson 68-304a* (holotype: MSC 216526! [not MSC 216528 or 289874]). Paratype: MEXICO, Baja California, Municipio de Ensenada, southwest of El Nuevo Rosarito, 28°36'40"N, 114°03'03"W, 100 m elevation, broad, dry arroyo with alkaline seeps, growing with *Distichlis spicata* (L.) Greene, *Juncus acutus* L., *Alleurolfea* sp., *Lycium* sp., and *Salicornia* sp., 2 April 2008, *Bell 458*, (BCMEX, MEXU, MO, RSA, UC, US).

Gramen perenne decumbens rhizomatosum stoloniferum ramis intravaginalibus secus stolonibus, 8–12 cm altum, ligulae pilis linea minuta dispositis, laminis 8–15 mm longis ad collo patentibus, in apicem pungentem sensim decrescentibus, ad faciem adaxialem parum flexis, pilis antrorsis secus margines et faciem abaxialem fascis vascularis medii ad et supra flexuram.

Sprawling, decumbent perennial with rhizomes and stolons, 8–12 cm tall, intravaginal branching along stolons, culms 1 mm in diameter, glabrous, sheaths open, glabrous, with tiny hairs along margins, ligules a minute line of hairs, blades 8–15 mm long, spreading at collar, narrowing gradually to pungent tip, with slight bend toward adaxial side, antrorse hairs along margins and along the abaxial side of the median vascular bundle at and above bend, male inflorescences a small panicle of racemes, inflorescences exerted above blade tips on peduncles of up to 1 cm, flattened pedicels 3–5 mm with toothed margins, 2–5 spikelets per inflorescence, 2–4 florets per spikelet, 1st glume 3 mm, 2nd glume 5 mm, both hyaline with a single nerve, lemmas 7–9 mm with

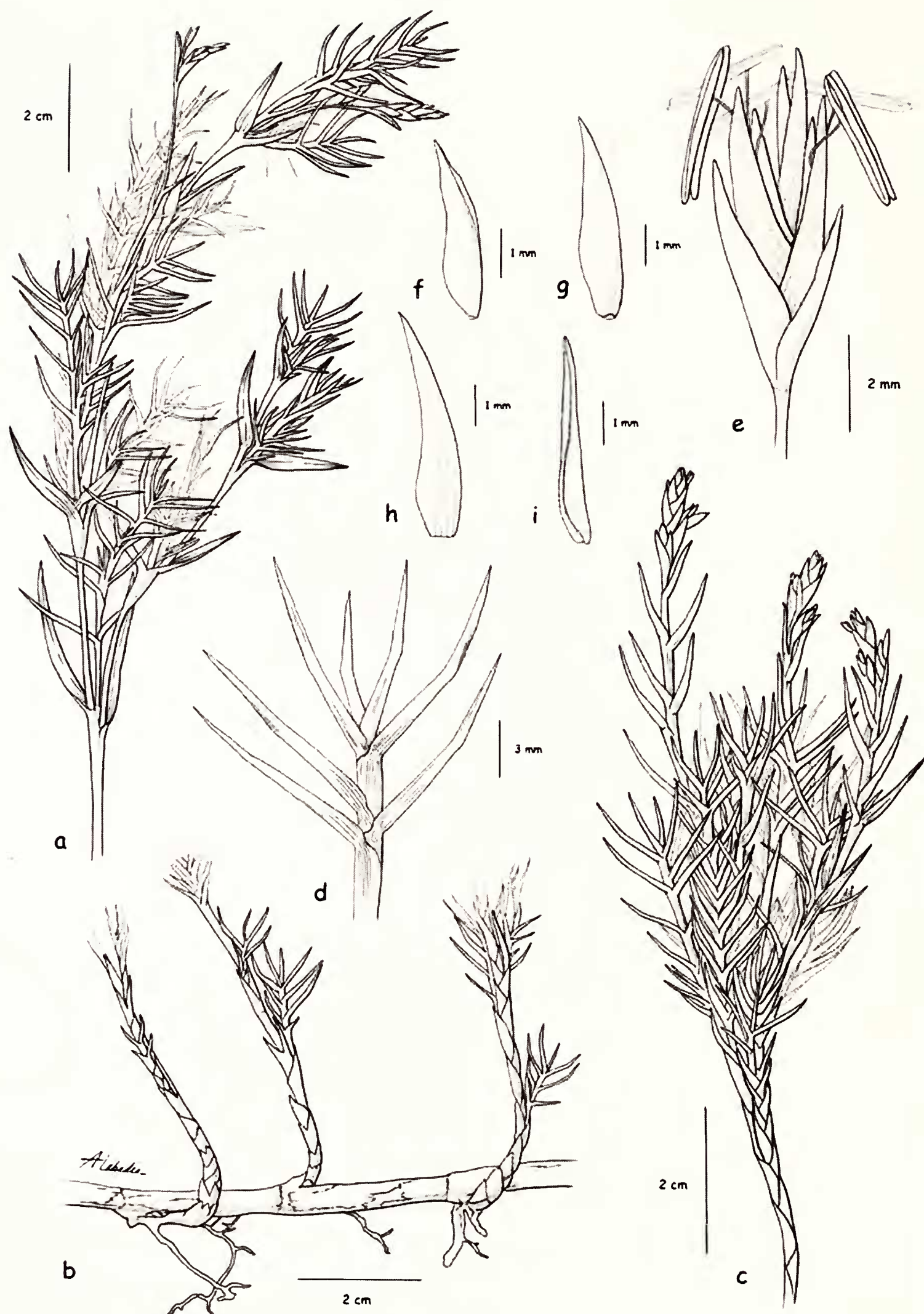


FIG. 8. *Distichlis bayaensis*. a. Female plant habit; b. Rhizome; c. Male plant habit; d. Detail of blades; e. Male spikelet; f. First glume from male spikelet; g. Second glume from male spikelet; h. Lemma from male spikelet; i. Palea from male spikelet. a and c from Stephenson 68-304a; b, d – i from Bell 458. Illustration by Amanda Labadie.

TABLE 2. COMPARISON OF CHARACTERS THAT CAN BE USED TO DISTINGUISH *DISTICHLIS BAJAENSIS*, *D. LITTORALIS*, AND *D. SPICATA*.

Character	<i>D. bajaensis</i>	<i>D. littoralis</i>	<i>D. spicata</i>
Blade length (cm)	0.8–1.5	<0.8	>2.0
Blade tips	narrow gradually to pungent tip	narrow abruptly to blunt tip	narrow gradually to blunt tip (rarely pungent)
Blade angle from culm	divaricate	divaricate	appressed or divaricate
Blade curve or bend	slight bend toward adaxial side	straight or slight curve toward abaxial side	generally straight
Glumes	present	absent	present
Male inflorescence exerted	yes	no	yes
Plant color	yellowish green	bluish green	bluish green

7–11 indistinct nerves, hyaline, palea slightly shorter than lemma, enclosed within lemma, anthers 2.5–3.5 mm, straw colored (some with purple tinge).

No fresh female inflorescences or caryopses were examined. Stephenson (1971) observed extensive grazing in the collection area and noted “only fragmentary grass specimens could be obtained”. He was not able to collect caryopses but provided observations of ovaries and stigmas. Table 2 gives characters that can be used to distinguish between *D. bajaensis*, *D. littoralis*, and *D. spicata*. A distinctive field character is a small bend near the middle of the leaf blade (Fig. 8d). Generally, at and above the bend, short, antrorse hairs occur along the median vascular bundle on the abaxial surface.

Future studies of *D. bajaensis* will focus on the total distribution of this species and the relationship of this species to the rest of *Distichlis*. At present, the species is known from a single large population that appeared to be all or predominately male. It is crucial to learn if other populations exist, the proportions of sexes in those populations, and their proximity to the Arroyo Rosarito population. Although Stephenson found male and female plants during his 1968 collection, John and Charlotte Reeder observed only male plants in 1979 (R. Felger, University of Arizona, personal communication). *Distichlis* species are capable of extensive vegetative reproduction via rhizomes and stolons and highly skewed sex ratios have been observed in many populations (e.g., *D. distichophylla*, Connor and Jacobs 1991; *D. spicata*, Freeman et al. 1976; Eppley et al. 1998). Even though vegetative reproduction occurs, the conservation status of *D. bajaensis* may well be extremely fragile with few or no female plants in existence.

The ITS phylogeny places *D. bajaensis* as sister to the remaining *Distichlis* (Fig. 1). If this is corroborated by future work, *D. bajaensis* will hold a phylogenetic position that is critical to investigating character development and evolution in *Distichlis* by enabling researchers to better

understand pleisomorphies vs. apomorphies in the genus.

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APPENDIX I

LIST OF TAXA SAMPLED

Taxa used as sources of DNA for the molecular phylogeny. See Bell and Columbus (2008) for additional details about *Distichlis* morphology and anatomy. Biogeographical abbreviations used in Figs. 1 and 2 are underlined. GenBank accession numbers (starting with EF or GU) appear in this order: ITS, *trnL-trnF*, *ndhF*. For a few specimens, the *trnL-trnF* sequence was not available and this is designated as 'NA'.

Allolepis texana (Vasey) Soderstr. & H. F. Decker. USA. TEXAS: *Bell* 240 (RSA), EF153021, EF156670, EF561646. *Bouteloua dactyloides* (Nutt.) Columbus. MEXICO. QUERÉTARO: *Columbus* 2329 (RSA), EF153026, EF156675, EF561647. *Eragrostis obtusiflora* (E. Fourn.) Scribn. MEXICO. MICHOACÁN: *Bell* 314 (RSA), EF196874, EF196902, EF561648. *Jouvea pilosa* (J. Presl) Scribn. MEXICO. JALISCO: *Bell* 247 (RSA), EF153057, EF156706, EF561649. *Distichlis acerosa* (Speg.) H.L. Bell & Columbus (= *Monanthochloë acerosa* (Griseb.) Speg.). ARGENTINA. LA RIOJA: *Bell* 389 (RSA), LR ARG, EF196897, EF196924, EF561671. CATAMARCA: *Bell* 392 (RSA), CA ARG, EF196898, EF196925, EF561672. *Distichlis australis* (Speg.) Villamil. ARGENTINA. RIO NEGRO: *Bell* 330 (RSA), RN ARG, EF196875, EF196903, EF561650. SANTA CRUZ: *Bell* 357 (RSA), SC ARG, EF196876, EF196904, EF561651. *Distichlis bajaensis* H.L. Bell. MEXICO. BAJA CALIFORNIA: *Bell* 458 (RSA), GU562862, NA, GU562863; *Stephenson* 68-304a (MSC), ITS Clones 1, 2, 3, 5, 6, 7, 8, 9, 10 GU562864, ITS Clone 4 GU562865, GU562867, GU562866. *Distichlis distichophylla* (Labill.) Fassett. AUSTRALIA. VICTORIA: *Cochrane* 1198 (MEL), VIC AUS, EF196877, EF196905, EF561652. SOUTH AUSTRALIA: 12 October 2003, *Walsh* s. n. (12 October 2003), SA AUS, EF196878, EF196906, EF561653. *Distichlis eludens* (Soderstr. & H.F. Decker) H.L. Bell & Columbus, (= *Reederchloa eludens* Soderstr. & H.F. Decker). MEXICO. SAN LUIS POTOSÍ: *Bell* 250 (RSA), SLP MEX, EF153077, EF156726; *Columbus* 4133 (RSA), SLPc MEX, EF196901, EF196928, EF561676. *Distichlis humilis* Phil. ARGENTINA. JUJUY: *Bell* 405 (RSA), JU ARG, EF196879, EF196907, EF561654. BOLIVIA. DEPARTAMENTO ORURO: *Peterson* 12833 (US), BOL, EF196880, NA, EF196908. *Distichlis laxiflora* Hack. ARGENTINA. BUENOS AIRES: *Bell* 367 (RSA), BA ARG, EF196881, EF196909, EF561656. CÓRDOBA: *Bell* 381 (RSA), CO ARG, EF196882, EF196910, EF561657. *Distichlis littoralis* (Engelm.) H.L. Bell & Columbus (= *Monanthochloë littoralis* Engelm.). USA. TEXAS: *Bell* 236 (RSA), coTX USA, EF153065, EF156714, EF561673. CALIFORNIA: *Bell* 260 (RSA), coCA USA, EF196900, EF196927, EF561674. *Distichlis palmeri* (Vasey) Fassett ex I. M. Johnst. MEXICO. SONORA: *Columbus* 3586 (RSA), SOc MEX, EF196883, EF196911, EF561658; *Felger* 91-39 (RSA), Sof MEX, EF196884, EF196912, EF561659. *Distichlis scoparia* (Nees ex Kunth) Arechav. ARGENTINA. RIO NEGRO: *Bell* 328 (RSA), RN ARG, EF196885, EF196913, EF561660. CHILE. VALPARAISO: *Bell* 374 (RSA), VA CHI, EF196886, EF196914, EF561661. *Distichlis spicata* (L.) Greene. USA. CALIFORNIA: *Bell* 231 (RSA), inCA USA,

EF153040, EF156689, EF561662; *Bell 259* (RSA), coCa
USA, EF196890, EF196918, EF561665. TEXAS: *Bell*
237 (RSA), TX USA, EF196887, EF196915, EF561663.
VIRGINIA: *Bell 290*, VR USA, (RSA), EF196892.
EF196920, EF561667. CANADA. BRITISH COLUM-
BIA: *Bell 277* (RSA), BC CAN, EF196891, EF196919,
EF561666. MEXICO. COAHUILA: *Bell 245* (RSA),

CO MEX, EF196888, EF196916, EF561664. ARGEN-
TINA. CHUBUT: *Bell 340* (RSA), CH ARG,
EF196893, EF196921, EF561668. CHILE. VALPA-
RAISO: *Bell 375* (RSA), VA CHI, EF196895,
EF196922, EF561669. PERU. REGION LAMBAYE-
QUE: *Columbus 3432*, (RSA), PERU, EF196896,
EF196923, EF561670.